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Requirement in Military Personnel

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INTRODUCTION

The purpose of this project is to develop quantitative estimates of 1) the amount of vitamin D produced by skin exposure to sunlight (Experiment 1, below), and 2) the amount of oral vitamin D that must be given to supplement solar inputs so as to achieve desired vitamin D levels in military personnel of differing races and skin pigmentation (Experiment 2, below).

This is the third annual report with respect to the above-referenced award. Although the award was made as of 1 October 2001, authorization to proceed was not received from USAMRMC until 15 July 2002. Hence this report, although technically covering the first three years of the award, describes work performed only from 15 July 2002 until submission of this report, i.e., a period of little more than two years.

BODY OF REPORT

Logistics. This project depends strongly upon a project manager with a minority background and good community contacts. We originally selected Lisa Auberry-Adams and put considerable effort into training her and obtaining the needed IRB certifications. Then, less than 12 months later she left us and we had to start the process over, this time with Tamicka Bradley. Tamicka helped greatly in pushing the project forward, but unfortunately, she, too, has left us for another research position. Since, at the time Tamicka left, we had recruited most of the targeted participant numbers, we decided not to replace her, but to rely upon existing Osteoporosis Research Center staff to complete the project.

Work Performed to Date: Experiment 2. The purpose of Experiment 2 is to quantify the serum 25(OH)D response (and its physiological correlates) to summer sun exposure in persons with a wide range of skin pigmentation. As of 30 September 2004 we had enrolled 71 individuals and had obtained both the first (i.e., late summer) and the second (late winter) measurements for most of them, as specified for Experiment 2. This number (71) is shy of our target of 80 participants with the shortfall being due to the loss of a project manager in two successive years at the peak of late summer recruitment. The racial and sex breakdown of the group recruited into Experiment 2 is as follows:

	Non-Hispanic Caucasian	Hispanic	African-American	Totals
Male	18	3	19	40
Female	13	2	16	31
Totals	31	5	35	71

For each of these subjects we have obtained the suite of specimens/measurements specified in the approved protocol, i.e., history of sun exposure by duration and clothing type; skin pigmentation by reflectance meter measurement; calcium absorption efficiency; measurements of the full set of hormones regulating the calcium economy [i.e., PTH, 1,25(OH)₂D₃, 25(OH)D], as well as blood vitamin D levels themselves, urine calcium excretion, and bone densitometry.

While degree of sun exposure varied, all participants were selected because they self-reported substantial mid-day sun exposure throughout most or all of the summer.

Results are still being analyzed, but we have sufficient data to permit several observations. Many of them (e.g., black-white differences) were already known from other studies; our goal in this project was to quantify them so as to develop better estimates of the amount of vitamin D producible in the skin in persons of varying color. This will allow (along with results of Experiment 1, below), development of evidence-based guidelines for vitamin D supplementation of DoD personnel. Findings to date (based on data from 65 subjects) are as follows:

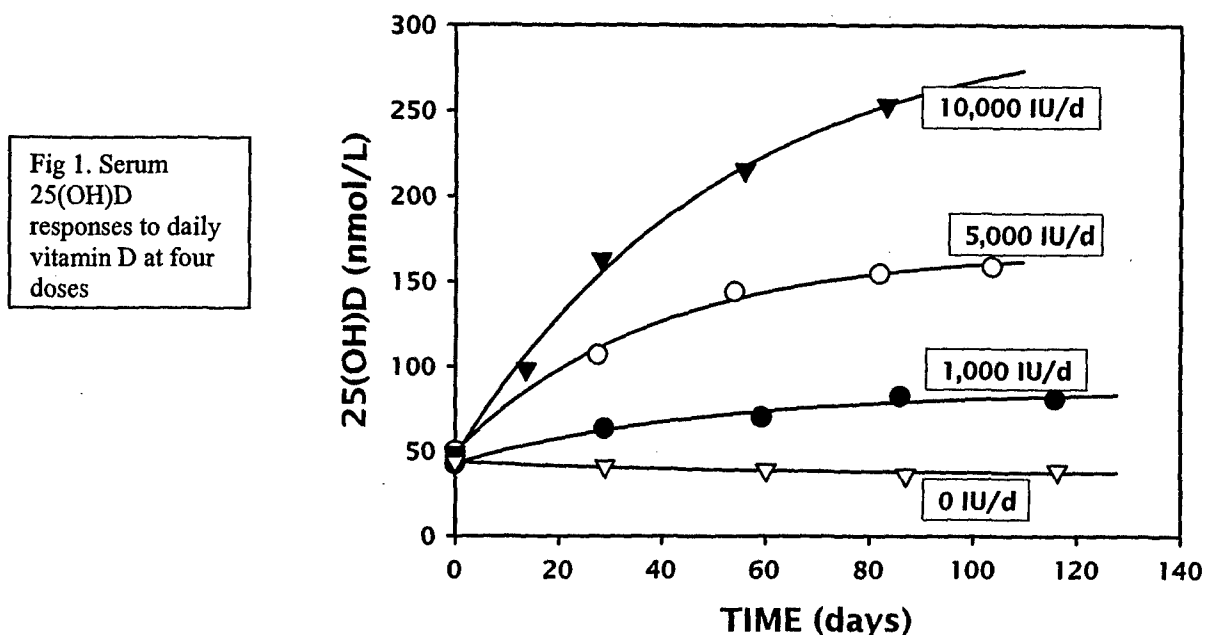
- African-Americans have lower serum 25(OH)D values than whites at both summer and winter measuring points. Although we have too few Hispanic subjects for a precise estimate, their data tend to be intermediate between blacks and whites.
- African-Americans, working outdoors, elevate their serum 25(OH)D levels to an extent not clearly different from whites (based on data available to date).
- Neither serum calcium nor urine calcium excretion differs between the late summer and late winter measurement times.
- Serum PTH rises significantly from late summer to late winter, indicating that the late winter level of 25(OH)D is physiologically inadequate (thus evoking increased PTH secretion).
- Calcium absorption efficiency is slightly (but significantly) higher at the late summer measurement point. This finding, as with the PTH difference, indicates that, by late winter, there is not only chemical evidence of vitamin D deficiency (i.e., low serum 25(OH)D), but physiological evidence as well (i.e., lower intestinal calcium absorption). Thus, steps to correct this inadequacy are likely to produce a benefit.

Work Performed: Experiment 1. The purpose of Experiment 1 is to quantify the ethnic differences (if any) in metabolism of known inputs of vitamin D₃. It is designed to be executed over the winter months when solar vitamin D input is minimal and total input can be controlled by the investigators through daily oral dosing of controlled quantities of vitamin D₃. Our plan was to split the project into two phases, studying doses of zero and 1000 IU/d during the first year of the project, and doses of 5,000 and 10,000 IU/d during the second year. We have largely completed specimen acquisition for this Experiment, and are currently analyzing both the specimens themselves, and the data derived therefrom. The description of results which follows reflects analyses to date, and may vary somewhat from what we report when the dataset is complete.

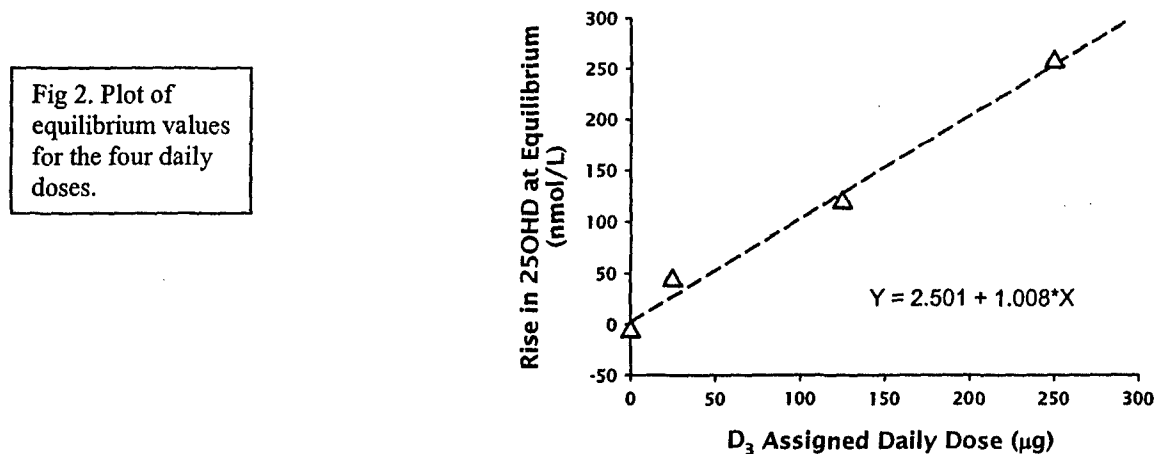
Racial and sex distribution of participants in Experiment 1 is as follows:

	Non-Hispanic Caucasian	Hispanic	African-American	Totals
Male	5	16	3	24
Female	13	9	48	70
Totals	18	25	51	94

These numbers are above our target figure of 80 enrollees. After eliminating data available to date from evident non-compliers, the principal data can be summarized in Figures 1 and 2, below.



The serum 25(OH)D plateau values reached on the right side of Fig. 1 are, as expected, dose-related. However, they do not differ appreciably from comparable values already reported (1) for an all-white sample. While we do not yet have sufficient data analyzed for a racial subgroup analysis, the general similarity of this total sample (~60% black) to our previous all-white sample, makes it likely that the metabolic handling of both vitamin D and 25(OH)D is essentially the same for both races. The zero dose curve of Fig 1 also shows graphically that serum 25OHD falls across winter without additional vitamin D input. However, at 1000 IU/d, the mean serum 25OHD rises to near 80 nmol/L, or close to the bottom of the healthy range.



In Fig. 2 we plot the relationship between the doses of vitamin D used and the plateau values achieved (see Fig.1). The slope of this relationship, as set forth in the Figure, is approximately 1 nmol/L/ μ g vitamin D/day, a value that is slightly higher than the value we had previously reported for an all-white group (i.e., 0.7 nmol/L/ μ g vitamin D/day) (1). This difference, if it persists on analysis of the full dataset, probably reflects the lower starting 25OHD values of this predominantly black sample. These starting values (as Fig 1 shows) are slightly below 50 nmol/L, or about half what might be considered ideal for that time of year (Autumn) in an unsupplemented population.

Work Plan for the Forthcoming Year. We anticipate spending most of the coming year on completion of analysis of acquired specimens, and on data clean-up, specimen reanalysis (as needed), statistical modeling and analysis, and report generation. Because of the late start on this project, we will probably be requesting an administrative extension without additional funds.

KEY RESEARCH FINDINGS

Key research findings (on the still incomplete dataset) are as set forth under Experiments 2 and 1, above. In addition to the presentation described in last year's progress report, a portion of these results were presented in poster form at the meeting of the American Society for Bone and Mineral Research in Seattle, WA, October 3, 2004. Copies of the poster and the associated Abstract are attached as Appendix I. Full analysis and publication must wait completion of analyses for all the subjects during this coming year.

REPORTABLE OUTCOMES

As noted in the foregoing, the reportable outcomes from this study will consist of 1) best quantitative estimates of skin production of vitamin D as a function of skin pigmentation and extent of skin exposure; and 2) best quantitative estimates of rate of utilization of vitamin D₃ as a function of race/ethnicity. Taken together, both will yield estimates of the quantity of vitamin D that must be given to military personnel to ensure maintenance of desired vitamin D status. Since much of the work is still underway, final quantitative estimates are not yet available. However, from the data produced so far, it seems safe to say that we will be able to produce the projected quantitative estimates. Moreover, secondary findings will become available and doubtless further such will develop as we accumulate more measurements. An example of such secondary data can be found in the Abstract and Poster attached as Appendix I.

CONCLUSIONS

None to date except as described above from partial analysis of the sample.

REFERENCES

1. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxy-cholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr* 77:204-210, 2003.
2. Barger-Lux MJ, Dowell MS, Heaney RP. A relationship between body composition and calcium absorption efficiency. *J Bone Miner Res* 19(Suppl 1):S302, 2004.

APPENDIX I

A Relationship Between Body Composition and Calcium Absorption Efficiency. M.J. Barger-Lux, M.S. Dowell, and R.P. Heaney, Creighton University, Omaha, NE

As part of an ongoing interest in sources of variation in Ca absorption efficiency (CaAbs), we examined its correlates in data collected for another study.

The 56 subjects (23 women and 33 men) were aged 20 to 51 y at entry. All reported ample summer sun exposure and limited non-solar sources of vitamin D. They classified themselves as black (n=23), white (n=29), or other (n=4). Median BMI was 27.1 kg/m² (inter-quartile range, 23.1 to 30.5).

Data were gathered at Visit 1 (after summer sun exposure, Aug. 23 to Sept. 21) and later, at Visit 2 (after winter sun deprivation, Feb. 1 to Mar. 15). We measured body composition (as % body fat by DXA), constitutive skin color (by use of a portable colorimeter with a standard color system), and CaAbs; fasting serum 25(OH)D, vitamin D₃, 1,25(OH)₂D, PTH, and Ca; and fasting 2h urine Ca-to-creatinine.

As expected, paired values for CaAbs were strongly related ($r = +0.702$, $P < 0.0001$). In these data, CaAbs was unrelated to skin color and to 25(OH)D, PTH, and 1,25(OH)₂D. However, CaAbs and % body fat were significantly and positively related at both visits, with a stronger relationship after controlling for BMI ($r = +0.425$, $P < 0.005$ and $r = +0.382$, $P < 0.01$, respectively).

We conclude that: (1) % body fat may be an important source of variation in Ca absorptive capacity (as an indicator of gut surface area?) and (2) further study is warranted.

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RESULTS

There were no significant differences between the two groups.

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1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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RELATIONSHIPS AMONG VARIABLES

- As expected, paired values in Gull-55 were strongly related to the P-00-04 Regression.

Figure 1 is a scatter plot showing the relationship between Percent Body Fat (x-axis, 0 to 100) and Cholesterol of VLDL 1 (y-axis, 0.0 to 0.8). The data points are categorized by age and sex: 12-17 F (open circles), 18-24 F (open squares), 25-34 F (open triangles), 12-17 M (filled circles), 18-24 M (filled squares), and 25-34 M (filled triangles). A regression line is shown with the equation $y = 0.007x + 0.1877$ and $r^2 = 0.1877$.

Detailed description of Figure 6: This scatter plot displays the relationship between body fat percentage and serum cholesterol levels. The x-axis, labeled 'Percent body fat', ranges from 0 to 50. The y-axis, labeled 'Cholesterol level (mmol/L)', ranges from 0.00 to 7.00. Three data series are plotted: Healthy controls (represented by open circles), HIV+ controls (represented by filled circles), and HIV+ treated individuals (represented by filled triangles). A solid regression line is drawn through the HIV+ treated data points, indicating a positive linear trend. Statistical values for this regression are provided as $r^2 = 0.89$ and $p < 0.0001$.

1. *Chlorophyll a* (Chl *a*)
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As a result of the above, the following is proposed as the definition of the *mean* of a fuzzy number \tilde{A} :

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[illegible]

DESCRIPTIVE DATA

[illegible]

SUBJECTS

Data are from 25 patients with positive results with computerized tests. All were female, with a mean age of 47 years (range 40-54), all had mild to moderate hearing impairment, and all had normal speech perception. They had been referred to the laboratory by their general practitioners. They had been

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

RELATIONSHIPS AMONG VARIABLES

- As expected, paired values in Gull-55 were strongly related to the P-00-04 Regression.

Figure 1 is a scatter plot showing the relationship between Percent Body Fat (x-axis, 0 to 100) and Cholesterol of VLDL 1 (y-axis, 0.0 to 0.8). The data points are categorized by age and sex: 12-17 F (open circles), 18-24 F (open squares), 25-34 F (open triangles), 12-17 M (filled circles), 18-24 M (filled squares), and 25-34 M (filled triangles). A regression line is shown with the equation $y = 0.007x + 0.1877$ and $r^2 = 0.1877$.

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 17. *Chlorophyll q* (Chl *q*)
 18. *Chlorophyll r* (Chl *r*)
 19. *Chlorophyll s* (Chl *s*)
 20. *Chlorophyll t* (Chl *t*)
 21. *Chlorophyll u* (Chl *u*)
 22. *Chlorophyll v* (Chl *v*)
 23. *Chlorophyll w* (Chl *w*)
 24. *Chlorophyll x* (Chl *x*)
 25. *Chlorophyll y* (Chl *y*)
 26. *Chlorophyll z* (Chl *z*)
 27. *Chlorophyll aa* (Chl *aa*)
 28. *Chlorophyll ab* (Chl *ab*)
 29. *Chlorophyll ac* (Chl *ac*)
 30. *Chlorophyll ad* (Chl *ad*)
 31. *Chlorophyll ae* (Chl *ae*)
 32. *Chlorophyll af* (Chl *af*)
 33. *Chlorophyll ag* (Chl *ag*)
 34. *Chlorophyll ah* (Chl *ah*)
 35. *Chlorophyll ai* (Chl *ai*)
 36. *Chlorophyll aj* (Chl *aj*)
 37. *Chlorophyll ak* (Chl *ak*)
 38. *Chlorophyll al* (Chl *al*)
 39. *Chlorophyll am* (Chl *am*)
 40. *Chlorophyll an* (Chl *an*)
 41. *Chlorophyll ao* (Chl *ao*)
 42. *Chlorophyll ap* (Chl *ap*)
 43. *Chlorophyll aq* (Chl *aq*)
 44. *Chlorophyll ar* (Chl *ar*)
 45. *Chlorophyll as* (Chl *as*)
 46. *Chlorophyll at* (Chl *at*)
 47. *Chlorophyll au* (Chl *au*)
 48. *Chlorophyll av* (Chl *av*)
 49. *Chlorophyll aw* (Chl *aw*)
 50. *Chlorophyll ax* (Chl *ax*)
 51. *Chlorophyll ay* (Chl *ay*)
 52. *Chlorophyll az* (Chl *az*)
 53. *Chlorophyll aza* (Chl *aza*)
 54. *Chlorophyll abz* (Chl *abz*)
 55. *Chlorophyll acz* (Chl *acz*)
 56. *Chlorophyll adz* (Chl *adz*)
 57. *Chlorophyll aez* (Chl *aez*)
 58. *Chlorophyll afz* (Chl *afz*)
 59. *Chlorophyll agz* (Chl *agz*)
 60. *Chlorophyll ahz* (Chl *ahz*)
 61. *Chlorophyll aiz* (Chl *aiz*)
 62. *Chlorophyll ajz* (Chl *ajz*)
 63. *Chlorophyll akz* (Chl *akz*)
 64. *Chlorophyll alz* (Chl *alz*)
 65. *Chlorophyll amz* (Chl *amz*)
 66. *Chlorophyll anz* (Chl *anz*)
 67. *Chlorophyll aoz* (Chl *aoz*)
 68. *Chlorophyll apz* (Chl *apz*)
 69. *Chlorophyll aqz* (Chl *aqz*)
 70. *Chlorophyll arz* (Chl *arz*)
 71. *Chlorophyll asz* (Chl *asz*)
 72. *Chlorophyll atz* (Chl *atz*)
 73. *Chlorophyll auz* (Chl *auz*)
 74. *Chlorophyll avz* (Chl *avz*)
 75. *Chlorophyll awz* (Chl *awz*)
 76. *Chlorophyll axz* (Chl *axz*)
 77. *Chlorophyll ayz* (Chl *ayz*)
 78. *Chlorophyll ayz* (Chl *ayz*)
 79. *Chlorophyll azz* (Chl *azz*)
 80. *Chlorophyll azaa* (Chl *aza*)
 81. *Chlorophyll abz* (Chl *abz*)
 82. *Chlorophyll acz* (Chl *acz*)
 83. *Chlorophyll adz* (Chl *adz*)
 84. *Chlorophyll aez* (Chl *aez*)
 85. *Chlorophyll afz* (Chl *afz*)
 86. *Chlorophyll agz* (Chl *agz*)
 87. *Chlorophyll ahz* (Chl *ahz*)
 88. *Chlorophyll aiz* (Chl *aiz*)
 89. *Chlorophyll ajz* (Chl *ajz*)
 90. *Chlorophyll akz* (Chl *akz*)
 91. *Chlorophyll alz* (Chl *alz*)
 92. *Chlorophyll amz* (Chl *amz*)
 93. *Chlorophyll anz* (Chl *anz*)
 94. *Chlorophyll aoz* (Chl *aoz*)
 95. *Chlorophyll apz* (Chl *apz*)
 96. *Chlorophyll aqz* (Chl *aqz*)
 97. *Chlorophyll arz* (Chl *arz*)
 98. *Chlorophyll asz* (Chl *asz*)
 99. *Chlorophyll atz* (Chl *atz*)
 100. *Chlorophyll auz* (Chl *auz*)
 101. *Chlorophyll avz* (Chl *avz*)
 102. *Chlorophyll awz* (Chl *awz*)
 103. *Chlorophyll axz* (Chl *axz*)
 104. *Chlorophyll ayz* (Chl *ayz*)
 105. *Chlorophyll ayz* (Chl *ayz*)
 106. *Chlorophyll azz* (Chl *azz*)
 107. *Chlorophyll azaa* (Chl *aza*)
 108. *Chlorophyll abz* (Chl *abz*)
 109. *Chlorophyll acz* (Chl *acz*)
 110. *Chlorophyll adz* (Chl *adz*)
 111. *Chlorophyll aez* (Chl *aez*)
 112. *Chlorophyll afz* (Chl *afz*)
 113. *Chlorophyll agz* (Chl *agz*)
 114. *Chlorophyll ahz* (Chl *ahz*)
 115. *Chlorophyll aiz* (Chl *aiz*)
 116. *Chlorophyll ajz* (Chl *ajz*)
 117. *Chlorophyll akz* (Chl *akz*)
 118. *Chlorophyll alz* (Chl *alz*)
 119. *Chlorophyll amz* (Chl *amz*)
 120. *Chlorophyll anz* (Chl *anz*)
 121. *Chlorophyll aoz* (Chl *aoz*)
 122. *Chlorophyll apz* (Chl *apz*)
 123. *Chlorophyll aqz* (Chl *aqz*)
 124. *Chlorophyll arz* (Chl *arz*)
 125. *Chlorophyll asz* (Chl *asz*)
 126. *Chlorophyll atz* (Chl *atz*)
 127. *Chlorophyll auz* (Chl *auz*)
 128. *Chlorophyll avz* (Chl *avz*)
 129. *Chlorophyll awz* (Chl *awz*)
 130. *Chlorophyll axz* (Chl *axz*)
 131. *Chlorophyll ayz* (Chl *ayz*)
 132. *Chlorophyll ayz* (Chl *ayz*)
 133.